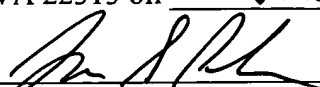


10/732,972 COFC

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Commissioner for Patents, P.O. Box 1450
Alexandria, VA 22313 on June 29, 2005

REQUEST FOR CERTIFICATE OF
CORRECTION UNDER 37 CFR 1.322
Docket No. UF-347XC1
Patent No. 6,878,933


James S. Parker, Patent Attorney

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Joshua J. Coon
Issued : April 12, 2005
Patent No. : 6,878,933
For : A Method for Coupling Laser Desorption to Ion Trap Mass Spectrometers

Certificate
JUL 08 2005
of Correction

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

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UNDER 37 CFR 1.322 (OFFICE MISTAKE)

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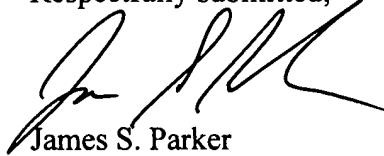
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Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,



James S. Parker

Patent Attorney

Registration No. 40,119

Phone No.: 352-375-8100

Fax No.: 352-372-5800

Address: P.O. Box 142950

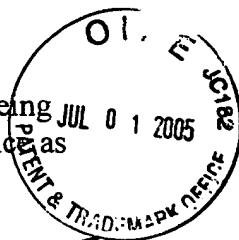
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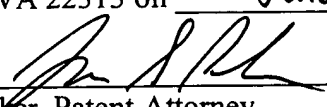
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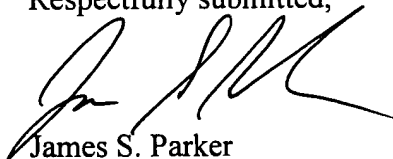
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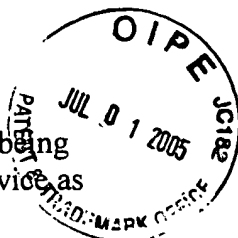
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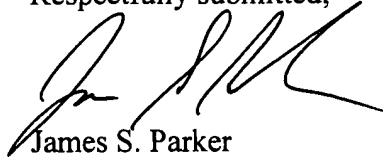
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CERTIFICATE OF CORRECTION

PATENT NO. : 6,897,933

Page 1 of 1

DATED : April 12, 2005

INVENTOR : Joshua J. Coon

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PATENT NO. 6,897,933

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Typically, QIT-MS systems are designed to use several levels of RF amplitude during a fixed ion injection period to optimally inject and store ions across a desired mass range, as each RF amplitude level used is optimal for injection and storage of ions within a certain mass-to-charge ratio (m/z) range. Prior AP-LD techniques used for injecting and storing ions into a QIT-MS have used laser pulses that are not at all synchronized with the RF amplitude levels of the QIT-MS. In contrast, in a preferred embodiment the subject method utilizes laser pulses that are synchronized to changes in the RF amplitude levels of the QIT-MS.

The subject invention finds applicability with both the traditional 3-dimensional quadrupole ion trap mass spectrometer (3D-QIT-MS) and the newer linear quadrupole ion trap mass spectrometer (L-QIT-MS). In a specific embodiment, the subject invention involves synchronizing laser pulses with the ion injection period of a L-QIT-MS. In a further specific embodiment, the laser pulses need not be synchronized with the ion injection period of the L-QIT-MS, but the laser pulses are caused to stop being incident on the sample during a portion, or all, of the time period outside the ion injection period of the L-QIT-MS. In another further specific embodiment, the laser pulses need not be synchronized with the ion injection period or the RF amplitude transitions of the 3-dimensional QIT-MS, but the laser pulses are caused to stop being incident on the sample during a portion, or all, of the time period outside the ion injection period of the 3 dimensional QIT-MS. By causing the laser pulses to stop being incident on the sample during a portion of, or all, of the time period outside the ion injection period of the 3D-QIT-MS or L-QIT-MS, sample waste can be reduced, or prevented. Causing the laser pulses to stop being incident on the sample can be accomplished by turning the laser off, blocking the laser beam from the sample, redirecting the laser beam away from the sample, or by other means known in the art. Preferably, the laser pulses are caused to stop being incident on the sample during the entire time period outside of the ion injection period of the 3D-QIT-MS or L-QIT-MS. In a specific embodiments the laser pulses can be stopped from being incident on the sample during at least a portion of the time period outside of the ion injection period, and preferably the entire time period outside of the ion injection period, of the L-QIT-MS, and the laser pulses incident on the

In a preferred embodiment of the subject invention, the AP-LD-QIT-MS interface has lasers that operate with high repetition rates (~ 100 Hz), and the ion pulses they generate are triggered to correspond with changes in injection RF levels so that the entire mass range can be consistently injected into the ion trap scan after scan.

5 By controlling the pulse frequency of the laser (e.g., from 10Hz to 100Hz), synchronously triggering the laser pulses with the changes in RF amplitude levels of the QIT-MS, and timing the laser pulses to the injection portion of the QIT-MS scan, sample consumption is reduced, the duty cycle is improved, and scan-to-scan reproducibility is improved, and the resultant analysis can be performed with higher precision across the
10 entire mass range. The reduction in sample consumption is accomplished by turning off the laser pulses during the portion of the QIT-MS scan which is not related to the injection of the ions. These portions of the QIT-MS scan include, for example, the mass analysis portion, pre-injection, post-injection, multiplier rise time, and post-scan time. The improvement in duty cycle results from the reduction of the QIT-MS injection period
15 (e.g., from 200 ms to 40 ms) This is enabled by eliminating the period between when the generation of ions from one pulse ends to the time of the next pulse. The improvement in scan-to-scan reproducibility is due to having injection and storage of ions into the QIT-MS uniformly in each RF amplitude subperiod.

20 In a specific embodiment, the pulse frequency of the laser is between about 1Hz and about 10Hz. In another embodiment of the subject invention the pulse frequency of the laser is between about 10Hz and about 100Hz. In yet another embodiment of the subject invention the pulse frequency of the laser is between about 100Hz and about 1000Hz.

25 The subject invention can be applied to a variety of techniques incorporating incidenting laser pulses on the sample to achieve atmospheric pressure laser desorption (AP-LD). Such techniques include, but are not limited to atmospheric pressure matrix assisted laser desorption ionization (AP-MALDI), atmospheric pressure laser desorption/ionization from porous silicon (AP-DIOS), and laser desorption-atmospheric pressure chemical ionization (LD-APCI).

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Further, with knowledge of the temporal ion pulse width produced from a desorption event, the laser repetition rate can be optimized. In a specific embodiment, the laser repetition rate can be approximately $1/T_{IP}$, where T_{IP} is the ion pulse width. For example, in a specific embodiment incorporating LD-APCI, an ion pulse width of ~ 10 ms was achieved. In this case, operation of the laser at a repetition rate of 100 Hz ($1/10\text{ms} = 100 \text{ Hz}$) can produce an approximately continuous supply of ions for MS sampling. Accordingly, based on the ion pulse width, the laser pulse repetition rate can be selected, or adjusted, to produce an approximately continuous ion supply. In a specific embodiment of the subject invention, Automatic Gain Control (AGC) is utilized in conjunction with the AP-LD measurement. QIT-MS systems typically operate optimally when a set value of ions are injected into the device scan after scan. AGC is a method implemented to vary the ion injection period (time ions are allowed to enter the QIT) to provide a constant number of ions. The constant number of ions can be a target value preset by the user. AGC is typically utilized with an ion source capable of providing a continuous supply of ions, e.g., electrospray (ESI). Prior AP-laser desorption approaches have produced pulsed supplies of ions and, thus, AGC has not been utilized. By optimizing the laser repetition rate to produce an approximately continuous supply of ions, the subject method can allow operation of AGC and thus further extend the utility of the AP-LD methods. In a specific embodiment operation of the laser repetition rate is approximately 100 Hz and, therefore, sample consumption per time can be a factor of 10 higher as compared to an embodiment having a laser repetition rate of 10Hz. To reduce the rate of sample consumption, the subject invention can synchronize the laser pulses to the mass spectrometric scanning, such that, for example, the laser only fires during times of ion injection. In this way, sample waste is reduced or eliminated.

In a specific embodiment of the subject invention, the QIT-MS control software causes the mass spectrometer to monitor the temporal pulse width of the desorbed ions and automatically adjusts the laser pulse frequency to maintain production of a continuous ion supply. In specific embodiments, for example with respect to AP-MALDI, changes in the crystal structure of the matrix could change the temporal ion

pulse profile, such that automatic adjustment of laser pulse frequency can allow the subject invention to maintain an approximately continuous ion supply.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

Following are examples which illustrate procedures for practicing the invention. It will be apparent to those skilled in the art that the examples involve use of materials and reagents that are commercially available from known sources, *e.g.*, chemical supply houses, so no details are given respecting them. These examples should not be construed as limiting.

Example 1 — Enhanced Performance with Rapid, Synchronized Laser Pulses

Figures 1A and 1B illustrate certain advantages of the subject invention over the prior art. Figure 1A shows how, in accordance with the prior art, two consecutive laser pulses (vertical dotted lines) separated by 100 milliseconds (ms), representing a 10 Hz operation cycle of the laser, align in a random fashion with a 200 ms ion injection period of the QIT-MS. The 200 ms injection period of the QIT-MS is broken down into four 50 ms subperiods, each with a different RF amplitude level favoring the injection and storage of a different ion m/z range. As seen in Figure 1A, the laser pulses (vertical dotted lines) only occur during the second and fourth subperiods of the 200 ms injection period. The shaded areas following the laser pulses represent the amount of time during which ions are being generated following the laser pulse. As can be seen from Figure 1A, no ions are generated during the first or third subperiod. Therefore, the ions m/z 's that are favored during the first and third subperiods are not appropriately represented in the QIT-MS measurement. In addition, there is much time between the end of the first shaded area and the next laser pulse, during which no ions are being generated for injection and storage.

Figure 1B shows how, in accordance with the subject invention, four consecutive laser pulses (vertical dotted lines) separated by 10 ms, representing a 100 Hz operation

Under continuous scanning, the ion-trapping period was varied from 0 to 100 ms in small increments. Afterwards, 10 scans, each composed of 10 single-shot mass spectra, were averaged from each ion trapping period segment and the absolute signal intensity of the spiperone protonated molecule was plotted vs. the magnitude of the ion trapping period (Figure 2). Note the AP-MALDI generated signal was multiplied by a factor of 90. In both cases, ions were first detected at a trapping period of ~ 6 ms with the signal increasing as the trapping period was lengthened. Extending the trapping period past 30 ms did not show significant increases in ion signal.

Polynomial curves were fit to each data series and are also shown in Figure 2. Using the equation from each curve, ion intensities were calculated for ion trapping periods from 0-30 ms in 1 ms increments. The magnitude of change between each trapping time was calculated and plotted in Figure 3. This plot represents the temporal ion flux during LD-APCI and AP-MALDI and demonstrates that the majority of the ion flux occurs during a 10 ms period for both methods.

In each case the earliest ion-trapping period that ions were observed was between 5 and 6 ms, which is also the period that carries the greatest ion flux. The 5 ms delay can be attributed to transport time, i.e., for any given set of AP-LD parameters there exists a fixed time required for ion transport from AP into the ion trap. Following that period, the largest flux of ions arrives at the ion trap during the following millisecond, after which ions continue to be formed over the course of the next 20-25 ms, but the signal continuously decays.

For AP-MALDI, a process that accomplishes desorption and ionization in a single-step rather than two as in LD-APCI, it is surprising that the temporal ion flux occurs on about the same time-scale. But since the chemical ionization is occurring at AP, where protonation reactions will occur on a rapid time-scale, ion transport is evidently the lengthiest step. In either case, the bulk of the ion signal arrives over a relatively short 10 ms period. Therefore, ion pulses on the order of 10 ms, spaced 100 ms apart (10 Hz laser repetition rate) during asynchronous AP-LD experiments, can hardly be considered a continuous source of ions as previously suggested (Laiko VV, Taranenko NI, Berkout VD, Musselman, Doroshenko VM, 2002, *Rapid Communications in Mass*

Spectrometry 16:1737-1742). Generation of a continuous ion beam would require sampling at repetition rates of 100 Hz (1 pulse every 10 ms). However, sampling this rapidly is simply not compatible without synchronization because of rapid sample consumption.

5 With this data in mind, consider the following typical AP-LD scenario: a 200 ms ion trapping period for which AP-LD is to be performed asynchronously with a laser operating at 10 Hz on a QIT-MS. Let us assume that all steps comprising the scan function, aside from ion injection and mass analysis, (e.g., pre-injection, post-injection, multiplier rise time, post-scan time, etc.) consume ~ 35 ms. We shall also assume that we
10 wish to analyze across a mass range of 500 – 2000 m/z, a typical mass range for applications such as tryptic peptide analysis. With the normal scan-rate of 0.18 ms/amu, scanning would consume ~ 270 ms. The entire scan would persist 505 ms, with ion injection accounting for only 200 ms; of those; thus, the correlating duty cycle would be approximately 40% at best, putting only 1 or 2 of the 5 laser pulses to analytical use.
15 Moreover, as described above, the majority of the ions are generated for only 10 ms following the laser pulse and, assuming 2 laser pulses occurred during ion injection, the resultant duty cycle would be ~ 4%. Further, to perform MS/MS, one of the most distinct advantages of the AP-LD-QIT-MS experiment, the duty cycle will drop even lower since additional time will be added to the scan function for ion isolation and fragmentation.

20 Besides the significant waste of sample in asynchronous AP-LD, ion injection is also a concern. Contemporary ion trap instruments with AP inlets typically utilize multiple levels of RF during ion injection so that all masses throughout the scan range will be successfully injected and stored. For the 200 ms ion injection period discussed above, Figure 1A displays the four ion injection RF levels, with each lasting for ~ 50 ms.
25 Also shown as dashed lines are two laser pulses separated by 100 ms (10 Hz operation) with the ion generation period shaded. Note that the pulses are always separated by 100 ms, but without synchronization they randomly shift from one scan to the next within the 200 ms window. An unfortunate artifact of this random shifting is that the ion pulses are randomly injected into different RF environments from one scan to the next; hence, a
30 large number of averaged scans are needed to generate suitable spectra.